RESISTANCE OF CILIA TO CYTOLYTIC AGENTS

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In studying the hæmolysis of erythrocytes from a series of mammals, Rywosch ('07) found that the order of resistance to saponin was almost the reverse of that to hypotonic NaCl. He did not however offer an explanation. Hoeber in 1912 suggested that saponin might affect the lipoids, but that the hypotonic solutions attacked some other component of the cell. K. Meyer (reviewed by Port) noted that corpuscles rich in cholesterol were also most resistant to saponin. The protective action of cholesterol in a cholesterol-lecithin mixture against saponin has been demonstrated on artificial membranes, and several workers (reviewed by Port, '10) very early cited Abderhalden's analyses of erythrocytes in support of the view that cholesterol is the chief factor in determining saponin resistance. Hoeber ('08 and '14) used several hypotonic salt solutions, and found that the phosphates were least injurious if used alone, but if mixed with saponin became the most injurious of the series. He interpreted this as due to the action of PO_4 on the cell colloids, but not until 1914 did he offer the following more elaborate theory. A cell protein which normally is stabilized by PO_4, will, after undergoing a change in sign (as by the action of acids), be destroyed by PO_4. Saponin produces such a change in sign of the corpuscles and therefore attacks especially cells rich in PO_4. This may explain the fact that a cell resistant to hypotonic NaCl is relatively susceptible to saponin.

The present experiments were undertaken to find whether this curious reversal of resistance occurs in cells of another sort (ciliated cells of various marine invertebrates), and to compare the effect of hypotonic NaCl with that of a hypotonic solution which is at the same time balanced, *e. g.*, dilute sea water.

The ciliated gill filaments of several molluscs and the ciliated larvæ of echinoderms, molluscs and annelid worms furnished

material for the experiments. The gills were divided into narrow strips of one or two filaments. The larvæ were generally heliotropic and so could easily be secured in large numbers in very little water. Not all the larvæ were fully normal: *Echinarachnius*, *Arenicola* and *Asterias* were the most variable in vitality. All of the solutions used (except of course NaCl) were made up in sea water rather than in distilled water and were never kept more than eight hours: not over four hours in the case of volatile substances. The experiments were carried out in stoppered test tubes which for higher temperatures were floated in warm water. The temperature of the tubes was read during every experiment. Each day's results were recorded on a separate slip of paper. The summary given below is an average of all observations and where these vary because of temperature, difference in viability (or other experimental error), the order of susceptibility has been verified by reference to the separate experiments in which the conditions must have been the same. The results obtained are as follows:

In comparing the resistance to saponin with that to hypotonic NaCl it is clear that although there are some irregularities (which are starred), the general order is the same. This is quite unlike the sharp reversal of order obtained with erythrocytes in saponin and in hypotonic NaCl. The order of cilia resistance in

TABLE I.

Saponin (per cent. represents dilution of a stock solution of 0.5 per cent. in sea water).

	20–23° C.							
Per Cent.	.25.	.5.	1.	5.	10.	30.	50.	100.
Echinarachnius gastr.....	8	5	5	2				
Arbacia 1 d.....		10	4	< 4				
" 2 d.....		10	8		3			
" 3 d.....	20	10+	6		3			
Echinarachnius pluteus.....			11	4	2			
Arenicola 2 d.....			15	3	4	3		
Chætopterus 1, 2 d.....			22	6	5	3		
" 3 d.....			15+	16	8	4		
Nereis 1 d.....			40+	12	8	5	3	
" 2 d.....			45	19	9	8	4	3
" 3 d.....			50		8	8	6	
Asterias 1–10 d.....			30+	60				80+
Cumingia 1, 2 d.....			30+					30+

Hypotonic Sea Water.

Per Cent.	20–23° C. 40.	35.	30.	20.
Echinarachnius plut.	30	15	5	< 2
" " gastr.	<30	15	5	< 2
Arbacia 1 d.		20+	$4\frac{1}{2}$	2
Arbacia 2 d.				
" 3 d.			7	2
Arenicola 2 d.	30+		8	5
Chætopterus 1, 2, 3 d.			20	9
Nereis 2 d.	30+	20+	12–22	8
Asterias 1–10 d.			18	9
Cumingia			30	22

Hypotonic NaCl (stock solution).

Per Cent.	20–23° C. 40.	30.	20.
Arbacia	11	8	
Arenicola	25+	5	
Echinarachnius	15+	< 2	
*Asterias 6 d.	15	18	15
**Cumingia*		25	15
Chætopterus	25	27	
Asterias 2, 7, 8 d.	7	25	10
Asterias 1 d.	15	30	15
Nereis		30	4

TABLE II.

Saponin (per cent, are presents dilution of a stock solution of 0.5 per cent. in sea water).

Per Cent.	20° C. 10.	20.	30.	50.	100.
Mytilus			8	4	2
Pecten	15	15	12	10	4
Mya	20	20	10	8	8
Quahog	25+	20+	15	8	8
Razor clam	50	30	30	18	17
Modiola	50+	30	30	27	20

Hypotonic Sea-water.

Per Cent.	20° C. 20.	15.	10.	5.
Pecten	6	4		2
Mytilus	35	16	8	3
Mya	35	15	9	4
Quahog	40	20	14	4
Razor clam	45+	35	14	4
Modiola	45+	35	14	4

Hypotonic NaCl.

Per Cent.	20° C. 15.	10.
Pecten	<3	<3
Mytilus	5	<3
Mya	5+	4
Quahog		7
Razor clam		8+
Modiola		

hypotonic sea water is almost exactly the same as in saponin solution: there is in fact only one slight difference (*Echinarachnius plut.*) as against two in NaCl. Evidently hypotonic sea water has not quite the same action on the cilia as hypotonic NaCl, but the difference is not great. No data on the hæmolytic power of a hypotonic balanced solution are available for comparison.

The action of acids upon erythrocytes (Koeppe, cited by Rywosch) is a function of their H^+ concentration, but their action upon cilia of protozoa (unpublished experiments) and of the giant clam (Harvey) is correlated with other factors as well.

Still another evidence of the peculiarity of erythrocytes is found in temperature coefficients. They show with a rise in temperature increased susceptibility to acids and to chloroform (Rywosch) but not to saponin or to hypotonic NaCl (Kagan). As the following table indicates this is not the case with most of the ciliated cells.

TABLE III.

Saponin.

	20–23° C.					25° C.			30° C.	
Per Cent.	0.5.	1.0.	30.	50.	100.	1.0.	30.	50.	0.5.	100.
Echinarachnius pl.		11				3				
Arbacia 2 d.	10	15	3			3			8	
Chætopterus 2 d.		22	3				3			
Nereis 1 d.		40	5	3				3		
Cumingia 2 d.		30			30			50		
Asterias 2 d.		60+			80			20		
" 3 d.								25		30
" 6–10 d.								60		

Hypotonic Sea Water.

	20–23° C.			25° C.		
Per Cent.	35.	30.	20.	35.	20.	20.
Echinarachnius pl.	15	5	2	10	3	2
" g.	15	5	2	20	5	
Arbacia 3 d.		7	2	25	5	2
Nereis 2 d.	20	15	8			13
Asterias 2 d.		18	9	60	15	9
" 4 d.				30	7	
" 6–10 d.					12	10

TABLE IV.

Saponin.

	20° C.					30° C.				
Per Cent.	10.	20.	30.	50.	100.	10.	20.	30.	50.	100.
Mytilus			8	4	2		4			
Pecten	15	15	12	10	4	5	4	2	2	
Mya	20	20	10	8	8		13	3	$3\frac{1}{2}$	
Quahog	25	20	15	8	8		16	5	4	
Razor clam	50	30	30	18	17	11	8	5	$2\frac{1}{2}$	3
Modiola	50	30	30	27	20	15	12	6	$4\frac{1}{2}$	4

Hypotonic Sea Water.

	20° C.				30° C.	
Per Cent.	20.	15.	10.	5.	20.	15.
Pecten	6	4		2	3	3
Mytilus	35	16	8	3	30	10
Mya	35	15	9	4	40	13

Thus resistance of cilia to both saponin and hypotonic NaCl is lowered by increasing temperature instead of remaining constant as is the case with erythrocytes. The coefficients are higher in saponin than in hypotonic sea water and also vary considerably from one form to another. This is not the case with erythrocytes. However the action of acids upon various infusoria, as upon erythrocytes, is markedly altered by change in temperature.

A few experiments were made using chloroform, ether and acetone made up in sea water (in vols. per cent., chloroform 0.1–0.2, and 0.37 per cent.; ether 2–6 per cent., acetone 10 per cent.). The gill cilia showed the same order of resistance in these reagents as in hypotonic sea water. The larval cilia were more irregular, as the following list shows, though the order is not very different from that obtaining in saponin.

Chloroform.	Ether.	Acetone.
Echinarachnius p.	*Echinarachnius* g. & p.	*Echinarachnius*
" g.	*Arbacia* 1, 3 d.	*Arbacia* 2, 3
Arbacia 1 d.	**Cumingia*	" 1
**Asterias* 3–4	*Asterias* 3, 6–10 d.	**Asterias* 2–6, 10
* " 2	*Chætopterus*	**Cumingia*
Arenicola 2	*Nereis* 1, 2	*Chætopterus*
Nereis 2	**Arenicola*	**Arenicola*
Cumingia	*Asterias* 1, 2	*Asterias*
Asterias 6–10		

With erythrocytes Rywosch found in each of these reagents a quite different order, more irregular than was observed in the present experiments.

An adequate explanation of these results is impossible at present. Although detailed analyses of corpuscles have long been available, there are analyses of only two of the organisms used in the present experiments, made upon the eggs (not larvæ) of *Arbacia* and *Asterias* by Matthews. He found the *Arbacia* egg rich in cholesterol as compared with lecithin and the *Asterias* egg the reverse. If these lipoids alone determined resistance to saponin and to hypotonic NaCl, *Arbacia* should be more resistant than *Asterias*, but this is not the case. This may be due to a change in the cholesterol content as the egg develops into a larva. Certainly differences due to age occur in most of the forms, especially at times of great morphological change. How-

ever, until we have adequate analyses we cannot offer a definite explanation of the characteristic differences in the behavior of these ciliated cells toward reagents.

Summary.

1. Cilia which are resistant to saponin are generally resistant to hypotonic sea water and hypotonic NaCl. The reversal of resistance found with erythrocytes does not hold with cilia.

2. Relative resistance of cilia to hypotonic NaCl is not the same as resistance to hypotonic sea water, although the irregularities are not numerous.

3. Most of the cilia show a change in resistance with change in temperature. This is more marked in saponin than in hypotonic sea water.

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